

Table A.

(1.) Percentage stretching.	(2.) Ratio of weight specific resistance after stretching to that before stretching.
0·04	1·000000
0·04	1·000037
1·82	1·000711
4·7	1·002463
9·06	1·003776
16·63	1·007128
21·98	1·010363

Table B.

(1.) Weight applied in lbs. avoirdupois.	(2.) Ratio of weight specific re- sistance with weight <i>on</i> to that before applying weight.	(3.) Ratio of weight specific re- sistance with weight <i>off</i> to that before applying weight.
14	1·00000	1·00000
28	1·00487	1·00000
56	1·00131	1·00000
70	1·00185	1·00005
84	1·00237	1·00010
98	1·00283	1·00090

“The Action of Gravity upon *Bacterium Zopfii*.” By RUBERT BOYCE, M.B., M.R.C.S., Assistant Professor of Pathology, University College, London, and A. ERNEST EVANS, M.B., C.M., Glasgow. Communicated by Professor VICTOR HORSLEY, F.R.S. Received February 7,—Read February 23, 1893.*

(From the Pathological Laboratory of University College, London.)

[PLATES 1 AND 2.]

In May, 1892, Mr. Walter Spencer handed over to us the body of a cat in which he had discovered a double otitis media. Some of the pus was immediately inoculated upon gelatine, and two days later it was seen that the gelatine along the streak had liquefied, whilst the rest of the surface of the non-liquefied gelatine was covered by a

* Of the numerous photographs illustrating this paper, only five of the more typical ones are reproduced, namely, figs. 10, 12, 14, 23, 28. It has been thought better, however, to preserve the original numeration in the text, as the original photographs can be consulted if necessary.

regular growth of delicate white filaments. Re-inoculation proved the presence of more than one micro-organism, but we soon succeeded in isolating one which did not liquefy the gelatine, and which presented the exceedingly characteristic feather-like appearance well known to belong to *Bacterium Zopfii*. We were especially struck by the beautiful regular upward growth of the filaments upon the gelatine surface, for whether we placed the tubes with their mouth pointing upwards or downwards there appeared the same definite growth, provided the tubes were kept in a nearly vertical position. We accordingly immediately commenced an investigation, which we hoped might throw some light upon the meaning of this very definite symmetrical growth. With this end in view we first investigated the action of light, but whether we grew the bacterium in the light or in the dark, the symmetry remained the same. We now began to observe, however, that tubes which, after inoculation, were kept in a horizontal position exhibited an *irregular* growth. It therefore occurred to us to test the action of *gravity*, and we found in effect that revolving inoculated test-tubes slowly upon the clinostat* produced irregular growths, whilst *centrifugal force* induced, like gravity, a regular growth. *Bacterium Zopfii* was therefore negatively geotropic. These phenomena, viewed in the light of the more recent experiments upon the chemotactic and physiotactic properties of protoplasm—properties which appear already to throw very considerable light upon certain pathological problems—have led us to venture to record in detail our observations.

Bacterium Zopfii was accidentally discovered by Kurth† in the alimentary tract of the hen in 1883, and his description of the micro-organism, furnished from Zopf's laboratory, remains the standard one. In 1885 Professor Crookshank‡ obtained from the air in John's laboratory an organism which he named *Bacillus figurans* on account of its characteristically figured growth upon gelatine and agar; it proved to be identical with Kurth's bacillus. Lastly, as previously mentioned, we obtained our growth from the middle ear of the cat. These observations make it very probable that *Bacterium Zopfii* has a wide distribution, but whether it is pathogenic or not is still uncertain. We will, however, return to this point later on.

Bacterium Zopfii is pleomorphic; it is a slender organism of about the same diameter as *Bacillus anthracis*, and its segments are of very variable length. It occurs in the dissociated motile state, the elements being actively movable coccil, bacteroid, bacillary, and

* We desire to express our thanks to Professor Oliver for not only the use of this and other instruments, but also for his friendly criticism and advice during the research.

† 'Bot. Zeitung,' 1883.

‡ 'Lancet,' 1885.

twisted forms; the degree of spirillation in the latter motile forms, however, appears only very slight, and we have not, as yet, encountered the pronounced spirilla seen in *Cladothrix*. It occurs in non-motile filaments of great length, which may be unsegmented or segmented. A very striking feature of these filaments is their proneness to twist, to weave themselves into striking patterns, and to form aggregating zoögleaform masses, of equally curious shapes. The segments of the filaments may occur in the form of cocci, bacteria, bacilli, vibrios, spirilla, and spirochætæ and in the various double forms of these. Kurth grew the organism in broth and on gelatine, and obtained in the former the motile and long filamentous forms, whilst the twisted varieties appeared wanting: this we likewise have noted. In gelatine he found that it caused an imperceptible amount of liquefaction and grew out in a radiate manner, and formed characteristic spirals. Crookshank grew it upon agar, and obtained a feather-like growth which he figures; this, however, we have always failed to obtain upon that medium. Kurth drew attention to the sensitiveness of the organism to changes in temperature, to the hindering action of the air, and to the great necessity for plenty of oxygen.

The Growth of Bacterium Zopfii upon Gelatine.

For our experiments we have always employed the 10 per cent. gelatine possessing a neutral or faintly alkaline reaction, but we have found no appreciable difference whether the organism was grown on gelatine with faintly alkaline or acidic reactions. We use the ordinary culture-tubes, Petri boxes, and a method of plate glass culture which we have found exceptionally useful. This method consists in placing a small piece of moist cotton wool in the bottom of a test-tube large enough to hold the ordinary 1×3 inch micro-slide; the test-tube is plugged, and the whole sterilised in the steam steriliser at 120° for 20 minutes. When cooled, a thin uniform layer of sterilised gelatine is very readily spread over the surface of the slide in the test-tube, which remains horizontal, by means of the balloon pipette of Pasteur: there is no fear of contamination. The test-tube is plugged and capped to prevent drying, and the slide is then always ready for inoculation. When a streak culture has been made upon the gelatine surface, and it is desired to examine the growth, the slide is removed and placed in slightly diluted spirit for a few hours, to fix the growth and extract the salts. The slide is then carefully dried, stained for a few seconds in gentian violet, washed with Gram's iodine, and nearly decolorised in spirit, again dried, mounted in Canada balsam, and covered with a sufficiently long cover-slip. By this method there is the least possible disturbance of the growth, and all our micro-

photographs have been taken from plates so prepared. The organism can always, however, be readily examined upon these gelatine slides without fixing or staining. The method of cover-slip impressions we have found not applicable in our case, as the growth is firmly embedded in the gelatine; it is useful, however, in the case of agar. For reasons which will be given later, it is necessary to observe under the microscope the ordinary test-tube growths *in situ* in the gelatine. For this purpose we rapidly warm the test-tube to just produce liquefaction of the surface of the gelatine in contact with the glass, and then slide it out upon a glass plate; a cover-slip is placed over the surface of the growth, and it can then be examined. Another method which we have employed, and one again which does not lead to any disturbance of the growth, is the well known method of drop cultures, but employing gelatine instead of a fluid medium.

Fig. 1 is a photograph of a 36 hours' growth upon gelatine, the result of a streak inoculation (see also fig. 12). The tube was kept vertical. It is the characteristic growth, *i.e.*, lines passing outwards and upwards at an angle of about 45° : the appearance is typically pinnate. In connexion with these vertical streak cultures we have noticed this very curious phenomenon, that no matter how crooked our original streak may be, yet the growth seems to form a new perfectly median axis for itself, from which the rami start; further, if instead of a vertical streak we make three or four streaks upon the surface of the gelatine at right angles to the long axis of the test-tube, the growth still tends to form a median line. These facts, coupled with our microscopic observations, pointed out to us that we might possibly be dealing with a branching phase similar to that met with in *Cladothrix dichotoma*; and, indeed, if we refer to Zopf's* drawings of *Cladothrix* we do recognize a pinnate arrangement of the so-called false branches of the filamentous organism. Billet† has termed this branched state the "filamentous," meaning thereby the "état d'accroissement proprement dit de la plante;" he has described it in *Cladothrix*, *Bacterium parasiticum*, *B. laminariae*, *B. ureae*, *B. Balbiani*, and *B. osteophilum*. In *Bacterium Zopfii* we shall regard the branching growths as corresponding to the "filamentous phase."

We soon found that we did not always obtain the characteristic growth, even in the test-tube; not only had the degree of sloping, as mentioned in the commencement, a considerable effect upon its regularity, but the temperature also exercises a marked effect. We obtained the best growths in a temperature of about 21° C. kept constant. In this way a perfect growth might be obtained in 24 hours, whilst if that portion of the test-tube which corresponded

* Schenk's 'Handbuch der Botanik.'

† 'Contrib. à l'Étude de la Morphologie et du Développement des Bactériacées.' Paris, 1890.

to the back of the gelatine was painted black, growth was still faster and might cover the surface in 12 hours. On the other hand we found accidentally that a reflecting surface retarded the growth. These latter phenomena we attributed to differences of radiation.

When next we directed our attention to the gelatine cultures on glass plates and in Petri boxes, we encountered a still greater number of irregularities. Upon the glass slides covered with a thin layer of gelatine we never obtained a good negatively geotropic growth; the usual condition was the irregularly branched appearance seen in figs. 15 and 16 under a low power. For the reasons of this we are still at a loss, unless it be the increased resistance of the gelatine. In the Petri boxes with a thickness of gelatine varying from $\frac{1}{4}$ to 1 inch, or even more, we obtained some instructive results. We never succeeded in getting symmetrical pinnate growths on *the surface* comparable to those in test-tubes; but that we obtained evidence of geotropism is manifest from the photographs, figs. 2, 3, 4, 5. In figs. 2 and 4 vertical streaks had been made; in fig. 3, a circular streak; and in fig. 5, a cross. The time occupied in the formation of these growths was relatively very much longer than in the case of test-tubes; moreover the filaments upon the surface rarely possessed the delicate character of that obtained in test-tube cultures; they had, in fact, as we shall subsequently see, the character of zoögleaform threads. In fig. 6 a horizontal streak has been made, and although the glass dish was kept vertical, an irregular growth has extended for an equal distance both above and below the horizontal line; *Bacterium Zopfii* may, in fact, readily extend downwards upon a surface, but it always does so in irregular masses. These results disappointed us at first, until on closer examination we found that in every case parallel threads *passed into the gelatine* almost invariably at the common angle of 45° . These geotropic threads had formed just as quickly as those found on the surface of the gelatine in the case of test-tube cultures; indeed, so rapid is their growth into the gelatine that they may extend a distance of $\frac{3}{4}$ to 1 inch in 12 hours. The symmetrical ingrowing into the gelatine we had also observed in the case of test-tube cultures where the growth upon the surface had been retarded by lowness of temperature, whilst painting the test-tube black in the manner previously referred to appeared to still further increase the inward growth. Fig. 7 is a view of the *back* of an early growth to show the regular ingrowing tendency; but photographs of the more perfect cases are impossible owing to the extreme delicacy of the filaments; fig. 5 is a later stage of 7. In fig. 6 the ingrowing is partially seen, and, as fast as the growth extended upon the surface, fresh rami projected symmetrically into the gelatine. We noticed in some cases that the filaments, after having penetrated the gelatine

and come in contact with the glass surface, might branch and be deflected back parallel to the ingrowing rami.

Growth upon Agar.—As stated above, we have never obtained a symmetrical growth in or upon this medium with or without glycerine. The growth is that figured in photograph 10. It takes place slowly, and the margins are irregular, being formed of scattered clumps of the bacterium.

Growth upon Potato.—Fig. 9 represents a potato culture; the faintly yellowish-white growth is observed to have no definite geotropic arrangement. The potato, it will be observed, appears black; this has been produced by means of iodine, a method which we have employed with success in demonstrating delicate growths upon potato surfaces.

Growth in Broth.—In broth abundant flocculi are formed.

Growth in Animals.—We have recorded how this micro-organism was first discovered by Kurth in the alimentary tract of the hen; whilst in our own case it was present in very great abundance in the pus of both the middle ears of the cat. It is just possible that in the latter (present) case the microbe gained access to the ear by the Eustachian tube, and therefore that the bacterium was originally in the mouth. It is conceivable, therefore, that it may, in common with the numerous other *Cladothrix* forms, be of more frequent occurrence in the mouth than is suspected. We have, however, made no observation upon this point. We inoculated two rabbits subcutaneously without result, and in another case the third of a test-tube full of a broth culture was injected into the peritoneum with like lack of success. These few experiments, however, prove little.

The Action of Oxygen and Carbonic Acid Gas upon Bacterium Zopfii.—We have already brought forward sufficient facts to show how sensitive this micro-organism is to changes of temperature. Fig. 11 represents a three days old growth in a test-tube kept in an atmosphere of carbonic acid gas; there is simply thickening of the original streak. Control test-tubes which were placed in oxygen at the same time exhibited well marked symmetrical growths from 12 to 24 hours. Further, if the tube in which the growth had been inhibited by the CO₂ were transferred to oxygen, a symmetrical growth occurred, as in fig. 12.

*The Action of the Spectrum upon the Growth of Bacterium Zopfii.**—By means of a Zeiss sub-stage spectroscopie a large spectrum was projected through the micro-photographic camera and focussed upon a Petri box of gelatine, across which a streak inoculation was made. From our results so far with gelatine or agar we can only say that

* For assistance in these experiments we are indebted to Dr. Fakirji Surveyor, who, with one of us, is engaged in the study of the action of the spectrum upon the pathogenic organisms.

the growth does not appear to be affected by any of the colours. Some of those who have seen our results think there is more growth towards the blue end; others, on the other hand, towards the red end. We ourselves have come to no definite conclusion as yet.

Microscopic Characters of the Cultures in the various Nutrient Media.—The series of photographs which illustrate these points have been fixed and stained in the gelatine, as previously mentioned. We have divided them into three groups. The first group, figs. 15 to 20, represent the *filamentous phase* of the early growth; fig. 17, for example, representing the long segments observed in the rapidly growing pinnate filaments. Groups II and III quickly follow upon the first. Group II, figs. 21 to 23, we venture to call the *skein phase*, which we think corresponds to the *état enchevêtré* of Billet. Group III, figs. 24—31, we think represent the *zoöglea phase*. There yet remains a fourth group, which we have not represented, and in which the segments live an independent existence (as partly shown in fig. 18) and very often are freely motile. We would call this the *dissociated phase*, the *état dissocié* of Billet. We have in this system of group phase division, therefore, followed Billet.* Whether this is a reasonable method of division will have to be judged from our photographs, from certain peculiarities exhibited by the above-mentioned groups, and lastly and chiefly from further examples.

In figs. 15 and 16, Zeiss obj., mm. 75 without eyepiece, the original streak of inoculation and the branches which spring from it are seen. The streak presents interesting peculiarities. Very slight liquefaction of the gelatine may be observed in it, and then very commonly swarming obtains; as previously mentioned, the motile segments may vary very greatly in length. In the figures in question clumps of bacilli occupy the central streak. In some cases, however, long zoöglea-like twisted masses, like those seen in fig. 25, run straight along this streak, it thus appearing as if their direction had been determined by the inoculating needle. In other cases the central streak is a mass of round zoögleaform collections. It will be seen that the micro-photographs in the first group afford very little evidence of the negatively geotropic tendency of the filaments, only the slight amount depicted in fig. 15 being often seen. The thicker branches in figs. 15 and 16, and all the branches in figs. 24 and 25, have assumed the zoögleaform condition. The microscopic picture presented by the rapidly growing pinnate form differs very markedly from these; in it nearly all the fibres in the field run parallel to one another, the microscopic picture then quite coinciding with the microscopic appearance seen, say, in fig. 1 or 14. In the pinnate forms it is exceedingly difficult to trace the origin of the fibres in the central line as well as to follow out the mode of branching; this is

* *Loc. cit.*

chiefly owing to the filaments slightly burrowing into the gelatine. The filaments may be very long and unbranched, but when branching does occur it appears to be brought about as in *Cladothrix* by the deflection of a segment to one side and subsequent growth. Soon after they are formed the long slender rami exhibit a remarkable proneness to twist and to segment, and then to pass into the zoögleaform or, less commonly, into the skein phases. The twisting is very striking, and some idea of it can be gathered from figs. 19, 25, 26, 27, 28, 29, 30. In none of these photos is the spirilliform twist of single filaments shown; it is, however, exceedingly common; the spiral is nevertheless seen in the zoöglea phases, figs. 25, 26, 30. There are, therefore, twists in the horizontal plane, and spirilliform twists. Concerning these twists it appears very remarkable that the majority appear to turn *inwards*, that is, in the opposite direction to the hands of the clock. To this point we will return when discussing the geotropism. It will be gathered from the above and from the photos that the segments may exhibit the "*form phases*" long ago laid stress upon by Zopf and Lankester; we need not, therefore, dwell upon them. The spiral is a striking feature in all gelatine preparations, but is only slightly marked in agar, whilst we have not as yet observed it in broth; but, we may add, we have not examined a sufficient number of these latter specimens.

Skein Phases.—The meaning which we wish to convey in this name is set forth in figs. 21, 22, and 23. We lay no stress upon it, but the term is convenient, for it is the form which can, we think, always be seen upon agar and stiff gelatine. It will be seen that it is quite unlike the zoögleaform phase. The filaments composing it are not held together by a common cementing substance, but are formed by plaits taking place in the course of a filament, as seen in figs. 21 and 19. Fig. 23 from an agar culture will at once recall the medusiform appearance of the anthrax growth. Its formation appears to be very closely related to the *stiffness* of the medium upon which it is growing, and appears always to occur *upon the surface of the medium*; the skeins are usually moist, and to the eye present a glistening frosted appearance.

Zoögleaform Phases.—This is the phase one commonly sees upon gelatine, and its striking appearance is well represented in figs. 24—31. Zoögleas are said to result from either the aggregation of dissociated elements or from the multiplication of segments which remain where they are formed, being held together by a common cementing substance. Of the former mode of formation we have no experience, and we venture to think that it is not common. If one turns to the descriptions of the higher bacteria, the impression is gained that the zoöglea masses have resulted from local multiplication; that this is so in *Bacterium Zopfii* the photographs show, as

well as the experiment of mixing a small quantity of the bacterium in liquefied sterile gelatine and then pouring it into a Petri dish; in the latter case numerous zoögleaform colonies develop in the solid gelatine, and send out branches which ramify in all directions. The branching zoöglea of *Cladothrix dichotoma*, commonly known as *Zoöglea ramigera*, follows closely the arrangement and branching of the filamentous stage; similarly, the "linear, globose, arborescent, reticular, and tessellated aggregations" of the *Bacterium rubescens* of Lankester probably represent local cell aggregation. As our figures show, the mode of aggregation leads to various forms, but they usually possess one feature in common, and that is spirillation, and a large number may be aptly compared to the twisted strands of a rope. The segments of which they are composed are small bacteroid or coccil forms, and they appear to be formed by the deflection and division of the minute segments into which the original single filaments break up. They rapidly follow the filamentous growth in the symmetrical pinnate cultivations; like the filaments which precede them, they assume the geotropic position. They may extend great lengths and be quite unbranched, or branching may occur as in figs. 30 and 31. Like the filaments, they form in the gelatine; we have not observed them in agar or broth. In fig. 31 a sarcina-like arrangement of the segments is seen; this is of interest, because, as Billet has pointed out, the sarcina of the lower bacteria probably is the representative of the zoöglea phase of the higher bacteria.

Upon the Phenomenon of Negative Geotropism exhibited by Bacterium Zopfii.

If a tube of gelatine is inoculated with a streak culture and kept in a nearly vertical position, a regular pinnate growth will, in the majority of cases, be obtained, identical with fig. 1. If a series of such tubes are placed at intermediate positions between the vertical and horizontal, it will be found that the growths become more and more irregular as the horizontal is reached. These results we have repeatedly obtained since May, 1892. But apparent failures do occur: *e.g.*, if the gelatine is too stiff, an irregular growth results, as in the case of agar, although the tube is kept vertical; microscopic examination of these irregular growths shows the skein phase. Similarly, if the temperature is low the symmetry is greatly interfered with. Experiments in the same manner with Petri boxes give, when sloped, the appearance already noticed in figs. 2 to 7. The pinnate growth is, in these cases, not so striking on the surface as with test-tube cultures, but, as we have previously remarked, the filaments which penetrate the gelatine ran a perfectly parallel course upwards, at an angle of about 45° , *into the substance of the gelatine*; the same

phenomenon may be observed in test-tubes, especially when the growth upon the surface tends to be irregular. If the Petri boxes are kept horizontal, the surface of the gelatine is soon covered with a quite uniform growth, in which, if rami can be distinguished, they take no particular direction. If, instead of these boxes, glass plate cultures are made, and in which, therefore, the coating of gelatine is necessarily thin, a pinnate growth with rami at the angle of about 45° is never obtained. The chance of getting a symmetrical growth upon a large flat surface is therefore much less than in the case of a test-tube. The explanation is difficult. In the test-tube, the surface of the gelatine has a slight curvature, and one often notices that towards the edge of the Petri dishes, where the gelatine is also slightly curved, the growth is more regular. Inequalities on the surface of the gelatine do cause alterations in the direction of the rami, and in some cases a few of the branches on this account appear to take a positive geotropic position. Thus, in addition to temperature and consistence of substratum, there are other circumstances which may favour or retard a symmetrical growth.

When test-tubes containing streak cultures are placed upon the vertical disc of the clinostat, and revolved at rates between one revolution in 2 minutes to 1 in 1 hour, there is in the first place a partial absence of the delicate rami seen in vertically sloped tubes, and when rami are formed they grow in various directions. Fig. 13 shows the irregular growth which first started, and grew over the middle area of the gelatine, and then the formation at the periphery of delicate rami, which in this case are completely *horizontal*. If a Petri dish is substituted for the test-tube, the result depicted in fig. 8 is obtained, a mode of growth which offers a striking comparison to figs. 2—6. Our results with the clinostat have been invariably the same, and, therefore, accord with what Sachs* obtained with the higher plants when the disc of the clinostat revolved slowly. In his case the direction of growth was neither that induced by gravity nor by centrifugal force. Having repeated these experiments very many times, we next tried Knight's experiment upon the action of centrifugal force upon growing plants. In his paper which was communicated to the Royal Society in 1806, he showed that, if young plants were rotated at rates varying between 80 to 250 times in a minute, that the ascending axis of the plant was centripetal, and the radicle centrifugal. Great was our satisfaction when we found likewise that the rami of *Bacterium Zopfii*, which, from the preceding experiments, we considered negatively geotropic, took a marked centripetal course when rotated in the horizontal at the rate of about 240 revolutions per minute. We obtained, in fact, as fig. 14 shows, much more symmetrical and beautiful results than

* Würzburg, Med.-Phys. Gesellschaft, March, 1872.

in the case of gravity. The angle remains about the same, and on microscopic examination the rami are found in nearly all cases strictly parallel and unbranched.

Our experiments thus show that we get a pinnate growth of *Bacterium Zopfii* when the surface of the medium is kept in or near the vertical, or when the culture is rapidly rotated in the horizontal. That an irregular growth obtains when the gelatine surface is horizontal, or when it is slowly rotated in the vertical. That it is necessary in order to demonstrate the upward growth to have a suitable temperature and suitable medium, and that other factors, the nature of which we do not understand, probably likewise influence the symmetrical growth. We would also draw attention to the fact that on vertical or nearly vertical gelatine surfaces, an irregular growth usually spreads *downwards* (compare fig. 6) upon the surface, and that when a horizontal surface of gelatine is inoculated, extension of the organism takes place vertically downwards into the substance of the gelatine; but in neither of these cases are the well-defined rami present. The question now remains, is the force which induces the upward growth of the protoplasmic threads of *Bacterium Zopfii*, *gravity*? Rozanoff* brought forward a beautiful example of upward growth in one of the Myxomycetes, *Æthelium septicum*. He grew it upon a nearly vertical surface of filter paper through which a very slow stream of water must have been constantly passing, as the upper edge of the paper rested in water. He found that the fungus grew upwards. He repeated Knight's centrifugal experiment, and found the fungus centripetal; during this experiment the filter paper was kept moist with water, and therefore the latter must have tended to fly outwards. He grew the *Æthelium* upon a horizontal surface of paper, *across* which a slow stream passed from one side to the other, and he found that growth did not take place more against the stream than with the stream. He argued whether this was an example of negative geotropism or of growth induced by the *contrary stream of water*, and he concluded that *Æthelium* was negatively geotropic, but that the *contrary flow of water* acted like the force of gravity and of centrifugalism. To our minds this does not seem an illogical conclusion, but Strasburger maintained that it was an example of *rheotropism* alone. Rheotropism, as far as fluid is concerned, cannot enter into our case. Our tubes were always plugged with absorbent cotton wool, and were not capped, and we never noticed moisture upon the surface of the gelatine, and, in any case, the geotropic rami tend to very slightly or deeply penetrate the gelatine. We have stated that both in vertical and centrifugalised gelatine growths, rami tend to pass *deeply into* the gelatine. Now we have observed, when we have placed in the

* 'Mém. Soc. Imp. des Sciences, Cherbourg,' vol. 14.

glass dishes gelatine to the depth of 1 inch or more, that at the lower portion of the streak of inoculation the rami which penetrated the gelatine were very distinctly more horizontal than those higher up. This phenomenon is also very conspicuously marked in the case of *centrifugalised tubes*. Further, our great difficulty in connexion with centrifugalising gelatine is to keep it in position, for in a large number of our experiments the gelatine is forced to the bottom of the tube, and the cotton plug is often driven some distance into the tube. We can see, further, that the compression to which the gelatine is subjected increases towards the distal end of the tube. Towards the distal end, however, the rami tend to become more horizontal, so it seems very much as if the tension of the gelatine caused the increased deviation to the horizontal. Having gone so far, we may ask why the rami are not orthogeotropic. We have many times called attention to the fact that the delicate filaments tend always to dip under the gelatine, and it may possibly be that it is the resistance of the gelatine which causes the deviation from the vertical to the curious angle of about 45°. We may here mention that this fact of the tendency to penetrate the gelatine is in apparent contradiction to the fact, that, as our experiments showed, CO₂ hinders and O favours symmetrical growth. Now, as a matter of fact, very little growth is obtained from a *stab* culture, so, therefore, it appears necessary that *some portion* of the growth should be close to or freely in contact with the air, and this obtains in *streak* cultures. To return to the action of the resistance of the gelatine, we have called attention to the fact that, next to the pinnate form of growth, the most striking feature in the life of *Bacterium Zopfii* is the tendency to curl. Kurth attributes this fact to the resistance of the gelatine. Now the twisting and the geotropic position of the rami we find tend to go hand in hand; they both tend to disappear upon agar or hard gelatine. Zopf noticed the absence of twisted forms in fluid. We find, as has been above stated, that the majority of the twists are in a direction contrary to the hands of a clock; many of the photographs show this, and these were taken at a time before we had directed our attention to the nature of the twist. Amongst the higher plants the inward twist is the commoner, and various theories have been made to account for the twisting. If we examine the rami which penetrate the gelatine we note that if they tend to be curved they tend to be convex on the lower surface, and that thus we tend to have two surfaces, a dorsal and a ventral, and can imagine with the botanist a difference in the tension of the protoplasm on these sides of the filament. According to De Vries, whose paper from the Wurtzburg Institute we have not been able to obtain, geotropism is not an improbable factor in the production of coils (Sachs). Wortmann* also

* "Theorie des Windens." 'Bot. Zeitung,' 1886.

regards coiling as the result of geotropism, and a second factor which he terms rotating mutation. In our case the coils may be related to the resistance of the gelatine and the negative geotropism. But spirillar forms are well known throughout the microphytes; they are present in *Cladothrix* and many more lowly-organised forms, and are found abundantly in fluid. Do, therefore, the spiral forms which we have described as forming in the gelatine correspond to the free spiral forms of the above examples? If they do, and we have not sufficient evidence to say they correspond, then the forces concerned in their formation in the one case would be equally applicable to the other.

DESCRIPTION OF PLATES.

PLATE 1. FIG. 10.—Represents a vertical streak inoculation upon agar. The irregular growth is characteristic.

FIG. 12.—This represents a culture which was first grown in an atmosphere of carbonic acid, and the only growth obtained was the thick irregular streak seen along the centre of the tube. It was then transferred to oxygen, and a pinnate growth obtained, as in the figure.

FIG. 14.—A typical symmetrical growth obtained by centrifugalising the culture tube in the horizontal.

PLATE 2. FIG. 23.—Skein, or Medusa-like growth, obtained upon agar.

FIG. 28.—Zooglea threads, showing the phenomenon of twisting. Gelatine growth.

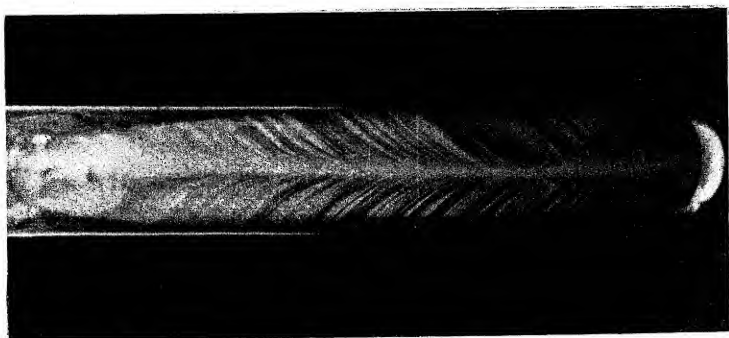


Fig. 14.



Fig. 12.

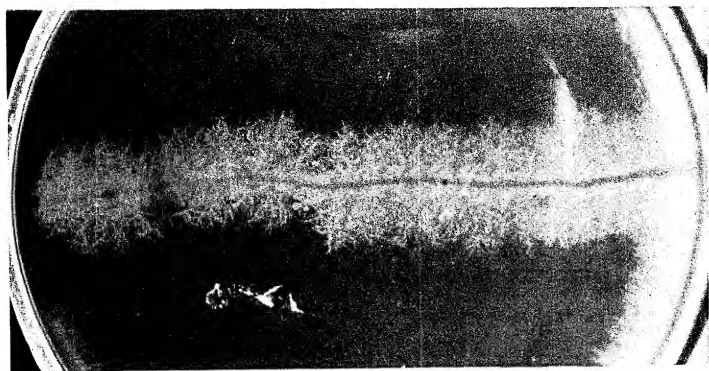


Fig. 10.

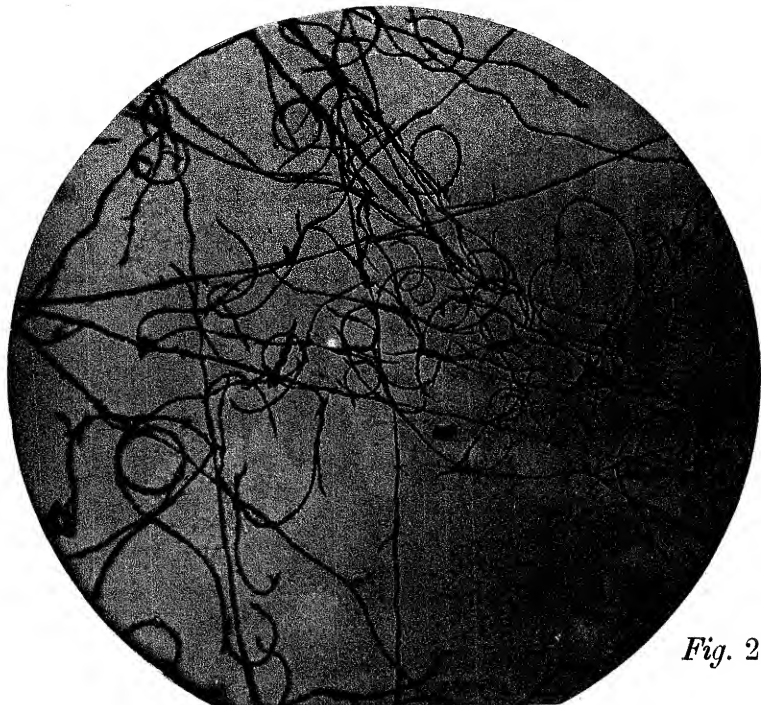


Fig. 28.

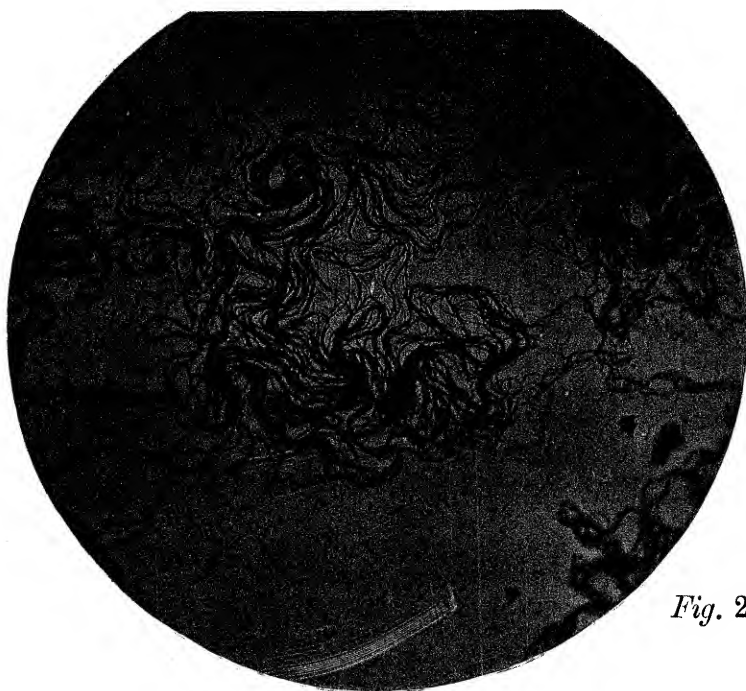


Fig. 23.



Fig 10.



Fig. 12.



Fig. 14.

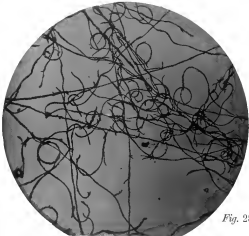


Fig. 28.

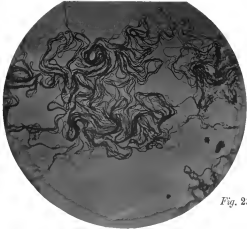


Fig. 23.